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A novel model for ex situ reperfusion of the human liver following subnormothermic machine perfusion

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Abstract

Machine perfusion-based organ preservation techniques are prudently transitioning into clinical practice. Although experimental data is compelling, the outcomes in the highly variable clinical donation-transplantation setting are unpredictable. Here, we offer an intermediate tool for pre-clinical assessment of human donor livers. We present a model for ex situ reperfusion of discarded human livers and report on its application in three human livers that have undergone subnormothermic (21°C) machine perfusion as an experimental preservation method. During reperfusion, the livers macroscopically reperfused in the first 15 minutes, and remained visually well-perfused for 3 hours of ex situ reperfusion. Bile production and oxygen consumption were observed throughout ex situ reperfusion. ATP levels increased 4.25-fold during SNMP. Between the end of SNMP and the end of reperfusion ATP levels dropped 45%. ALT levels in blood increased rapidly in the first 30 minutes and ALT release continued to taper off towards the end of perfusion. Release of CRP, TNF-α, IL-1β, and IL-12, IFN-γ was sustained during reperfusion. These findings support the use of this model for the evaluation of novel human liver preservation techniques.

Keywords

Organ Preservation; Biopreservation; Liver Transplantation; Machine Perfusion; Reperfusion; Ischemia

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DISCLOSURES

Dr. Uygun is inventor on pending patents relevant to this study (WO/2011/002926; WO/2011/35223) and Drs. Uygun and Bruinsma have a provisional patent application relevant to this study (MGH 22743). Dr. Uygun has a financial interest in Organ Solutions, a company focused on developing organ preservation technology. Dr. Uygun's interests are managed by the MGH and Partners HealthCare in accordance with their conflict of interest policies.

INNOVATION

Novel organ preservation methods have been studied in numerous models to date, including animal and discarded human organ models. Discarded human organs have proven useful in establishing proof of concept, however no models exist that provide a useful pre-clinical representation of reperfusion of the human organ. As novel organ preservation techniques are developed towards clinical studies, a pre-clinical human reperfusion model would enable evaluation of the technique in the uncontrolled setting of human organ donation. Moreover, a human organ reperfusion model would reveal unanticipated outcomes following reperfusion of inherently variable human organs, prior to translation from animal models to clinical studies. In this study, we report on a novel model of ex situ reperfusion of the discarded human liver, using diluted whole blood sourced from the liver donor. To our knowledge this is the first study that uses a simulated reperfusion to evaluate human livers following experimental preservation.

INTRODUCTION

Alternatives to static cold storage (SCS) of the liver have received significant interest as a means of expanding the currently limited donor organ pool. Machine perfusion (MP) techniques, in particular, have developed rapidly and are prudently being translated to clinical application[1]. These techniques aim to improve the preservation of the liver by offering a more supportive environment by providing a continuous flow of oxygen and nutrients through the organ, from hypothermic, to normothermic temperatures, including subnormothermic machine perfusion (SNMP)[2]. In animal models, benefits of MP have been shown over the full temperature spectrum. In these models, superiority over conventional SCS has been demonstrated using orthotopic transplantation[3–5] or a simulated reperfusion ex situ[6–8]. For a number of reasons clinical translation of these models should proceed prudently. Firstly, the controlled experimental environment is not representative of the true logistics of transplantation. Secondly, variability in human livers is sizeable and unpredictable. While experimental models are an adequate representation of a healthy organ, machine perfusion following the combination of donor factors (steatosis, age, nutritional status) as well as procurement factors (surgical technique, warm- and cold ischemia time, flush) may prove erratic when transitioning to the clinical application[9]. For this reason, we and other groups have performed MP of discarded grafts to learn the specific dynamics of human liver perfusion[10,11]. This provides insight into human liver function and injury during perfusion preservation, but omits valuable information on the effect on the graft after reperfusion and fails to completely capture ischemia/reperfusion injury (IRI). To date, no pre-clinical models for reperfusion of the human liver after machine perfusion preservation have been described. Herein, we present a procedure to model reperfusion ex situ using diluted autologous whole blood, which was conceptually and logistically tested using discarded human livers donated after cardiac death and preserved by SNMP.

MATERIALS AND METHODS

Experimental design

Discarded human livers were used for testing of the experimental model ($n = 3$). All grafts were donated after cardiac death (DCD), and were procured in the New England Donor Services (NEDS) region with consent from the donor's family for research use. Grafts were rejected for transplantation due to DCD status in combination with donor age ($n = 2$) or macrovesicular steatosis ($n = 1$). Livers were procured following standard procurement procedures for transplantation, described in more detail elsewhere[12]. Autologous blood was collected simultaneously with organ procurement, as described below. Liver and blood were packaged on ice and transported to our setting, while the SNMP was prepared. Target cold ischemia time, measured between cold flush and connection to the perfusion device was 240 minutes.

The Massachusetts General Hospital Institutional Review Board (IRB) and the New England Donor Services (NEDS) approved this study (No. 2011P001496) and all studies were carried out in accordance with IRB and NEDS approved guidelines.

Autologous blood collection and processing

Suction canisters used perioperatively for blood drainage were primed with 10^4 U of sodium heparin. Blood was collected following transection of the vena cava, and during the cold flush of the organs. Six liters of blood combined with University of Wisconsin solution could generally be collected. On arrival at our center, blood was processed to produce a clean cell concentrate. The solution was first filtered through a 90-pm mesh and subsequently centrifuged at 2,400 rpm. The erythrocyte and leukocyte layers were washed twice in 10% dextrose in 0.9% NaCl solution. A final hematocrit of 21% was achieved using type-matched fresh frozen plasma (FFP). The final volume of 1.5 L was finally supplemented with 100 U of insulin (Humulin), an additional 2,000 units of sodiumheparin, ceftriaxone 1 g/L. The pH was corrected to 7.30–7.50 using sodiumbicarbonate 8.4%.

Subnormothermic machine perfusion

Subnormothermic machine perfusion was performed following the concepts and methods described elsewhere[11], with the difference in the use of a commercial MP device (Liver Assist, Organ Assist B.V., Groningen, The Netherlands). Briefly, this involves 3 hours of MP at 21°C using oxygenated (95% O_2/5 CO_2) Williams' Medium E, perfusing the liver through the portal vein and hepatic artery at set pressures of 3 and 30 mmHg, respectively.

Ex situ autologous whole-blood reperfusion

Following machine perfusion, the liver was cold flushed with ice-cold Lactated Ringers (LR) solution over a period of 20 minutes to reflect sewing-in time. During this time the device was primed for reperfusion with the diluted whole-blood and set to 37°C. Oxygenators were gassed with 95% O_2/5% CO_2 and blood was sampled for adequate pH (7.3−7.5) and oxygenation. The liver was then connected to the MP system and pressure on the portal vein and hepatic artery were set to 6 mmHg and 60 mmHg, respectively, matching hemodynamics seen intraoperatively[13]. Reperfusion was continued for three hours, while

perfusion measurements were recorded and blood and tissue samples were taken and stored at −80°C before further analysis. Blood gas analysis was performed using a benchtop bloodgas analyzer (Rapidpoint 500, Siemens). Oxygen consumption was calculated from the difference between in and outflow dissolved and hemoglobin-bound oxygen content.

Functional and injury assessment

Alanine transaminase (ALT) release was determined in the blood using a point-of-care blood chemistry analyzer (Piccolo, Abaxis, Union City, CA). Levels of C-reactive protein (CRP), TNF- α, IL-1β, IFN-γ and IL-12 were determined in the blood using a multiplex bead array (Eve Technologies, Calgary, Canada). Absolute concentration of adenosine triphosphate (ATP) was measured in liver samples using a luminescence-based assay (Cell Viability Kit; Biovision). Nucleotides were extracted from ~1 mg of homogenized tissue, after which peak luminescence was determined. ATP concentrations were normalized to tissue protein using a BCA protein assay (Thermo Fisher Scientific, Waltham, MA).

RESULTS

Using the technical steps of organ procurement, preparation of the autologous whole blood and the liver, and priming of the machine perfusion device, we were successful in achieving a reproducible model of reperfusion. Collection of the blood from the organ donor reliably provided the required 1.5 L of diluted whole blood (21% hematocrit). Using this machine perfusion device, flow during SNMP was consistent with what has been previously observed with a different device (Fig. 1)[11]. Following SNMP and 20 minutes of cold-flush with LR solution, the livers were successfully reconnected to the blood-primed device for reperfusion.

Ex situ autologous whole-blood reperfusion

The livers macroscopically reperfused during the first 15 minutes, and remained visually well-perfused during the 3 hours of ex situ reperfusion (Fig. 2a). Flow in the portal vein was an average of 0.37 ± 0.16 mL/min.g liver in the first 10 minutes and gradually increased and plateaued at an average of 0.79 ± 0.16 mL/min.g liver at three hours (Fig. 2b). Bile production began nearly immediately in all cases and continued to flow constantly at an average rate of 7.0 mL/h throughout (Fig. 2c). Oxygen uptake, calculated from the difference in inflow and outflow dissolved and Hb-bound O_2, remained fairly consistent during reperfusion (~ 20 mL O_2/min.kg liver) (Fig. 2d). ATP levels increased 4.25-fold during SNMP (Fig. 3). Between the end of SNMP and the end of reperfusion ATP levels dropped 45%, from 3.64 ± 1.11 to 2.51 ± 0.262 nmol mg protein^{-1}. ALT concentrations in blood increased most rapidly in the first 30 minutes of reperfusion and ALT release continued to taper off until the end of reperfusion (Fig. 4a). Average ALT release into the blood at the end of reperfusion was 1155 ± 225 IU/L. Release of CRP, TNF-a, IL-1p, and IL-12, IFN-y was sustained during reperfusion (Fig. 4b–f).

DISCUSSION

Herein we present a methodology to model reperfusion of the human liver ex situ as a means to model IRI and study novel liver preservation techniques. Various liver machine perfusion techniques are currently transitioning into clinical application, including the first normothermic perfusion preservation modalities[14,15]. This translation is backed by preclinical reports in experimental models, which have proven promising by showing improved results after orthotopic transplantation in animal models using machine perfusion-based preservation techniques[5,16]. While these studies provide basic evidence for feasibility of the techniques, they do not factor in the complexities and unpredictability of human liver donation and transplantation. For instance, the logistics of human organ transplantation, including transport and timing, is far more complex than in a controlled experimental setting. Moreover, human livers exhibit a tremendous variation in organ characteristics[9], which can lead to unanticipated results when attempting to transplant these organs after machine perfusion. To this end we provide an experimental tool for pre-clinical modeling of reperfusion in human livers.

In stead of orthotopic transplantation, various experimental reports have used a simulated ex situ reperfusion in animal models[6–8]. Donor blood is collected and used in an ex situ normothermic perfusion set-up to model reperfusion instead of transplanting the liver. While this method does not fully capture the transplantation process, it has the advantage of being more controlled than transplantation as well as being more conducive to measurements, including flow dynamics, bile production and histological sampling. We have shown previously that diluted whole blood reperfusion accurately models reperfusion and analysis of leaked transaminases during simulated ex situ are predictive of actual transplant outcome[17].

To our knowledge report is the first ex situ reperfusion of a human liver following machine perfusion as a preservation method. Using whole human blood is complicated by the availability of human blood for experimental purposes. To overcome this, we procured autologous blood from the organ donor concurrent with the organ procurement. The use of autologous blood omits immunological responses of allogeneic transplantation, which is rare and arguably negligible in ABO-compatible liver transplants. The presence of leukocytes in the blood does however retain reperfusion-induced inflammatory response, which is more prominent in the early stages of transplantation. Moreover, consistently using blood from the organ donor improves uniformity between reperfusions. We are uninformed, however, of what effects heterologous blood would have on the parameters studied and this should be taken into consideration. In addition, the extensive manipulation of the blood may result in activation or alteration of the function of present immunocompetent cells.

The technical steps outlined here to procure blood from the liver donor consistently enabled us to produce the required amount of diluted whole blood, with a target hematocrit of 21%, which was achieved using unused FFP from our institution’s blood bank. The reperfusion was performed on a commercial liver perfusion device, which is designed for a full-range of temperatures. Our results using three discarded human livers show a homogeneous reperfusion of the organ and a consistent flow through both the hepatic artery and portal vein

following the initial reperfusion and rewarming of the graft. Moreover, bile production and oxygen uptake were stable during the three hours of simulated reperfusion, demonstrating the stability of the system.

In various reports we have emphasized the importance of energy metabolism for transplant outcome[4,9,11,12,18] We present the ATP levels at various stages of the perfusion-reperfusion process and reiterate the stark increase we have seen after SNMP of both cold and warm ischemic livers[4,19]. Interestingly, 3 hours of reperfusion of the livers following SNMP is not able to restore ATP levels to those seen after SNMP. We explain this by a likely significant drop in ATP during the modeled 'sewing in' time, during which the liver is ischemic, as well as the injurious effects of reperfusion attenuating energetic recovery. The relatively short reperfusion period does not allow for interrogation of ATP levels later in the reperfusion process, nor the later stages of IRI. We report on the release of injury markers here and show a sustained release of ALT and CRP as well as various inflammatory cytokines, which is expected during reperfusion after MP[20]. It is important to note that the livers studied here were found to be of insufficient quality for use in transplantation, and that machine perfusion and simulated reperfusion of higher quality livers may give different results.

In conclusion, this report outlines for the first time a procedure for simulated reperfusion of the human liver using autologous whole-blood obtained from the organ donor, which was evaluated in discarded human livers preserved by SNMP. The experimental technique offers a pre-clinical tool for assessing human livers, facilitating the translation of machine perfusion-based liver preservation into clinical practice.

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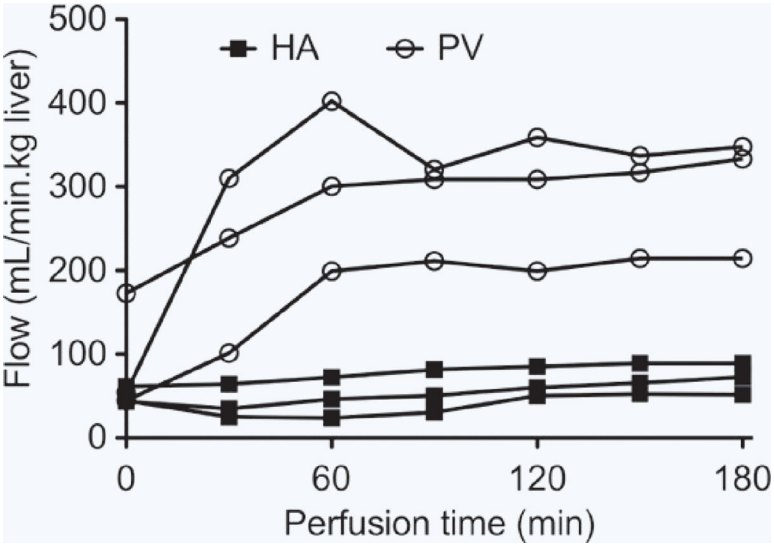

Figure 1. Perfusion dynamics during SNMP.

Flow rates during SNMP as measured on the portal vein and hepatic artery. Lines represent individual livers.

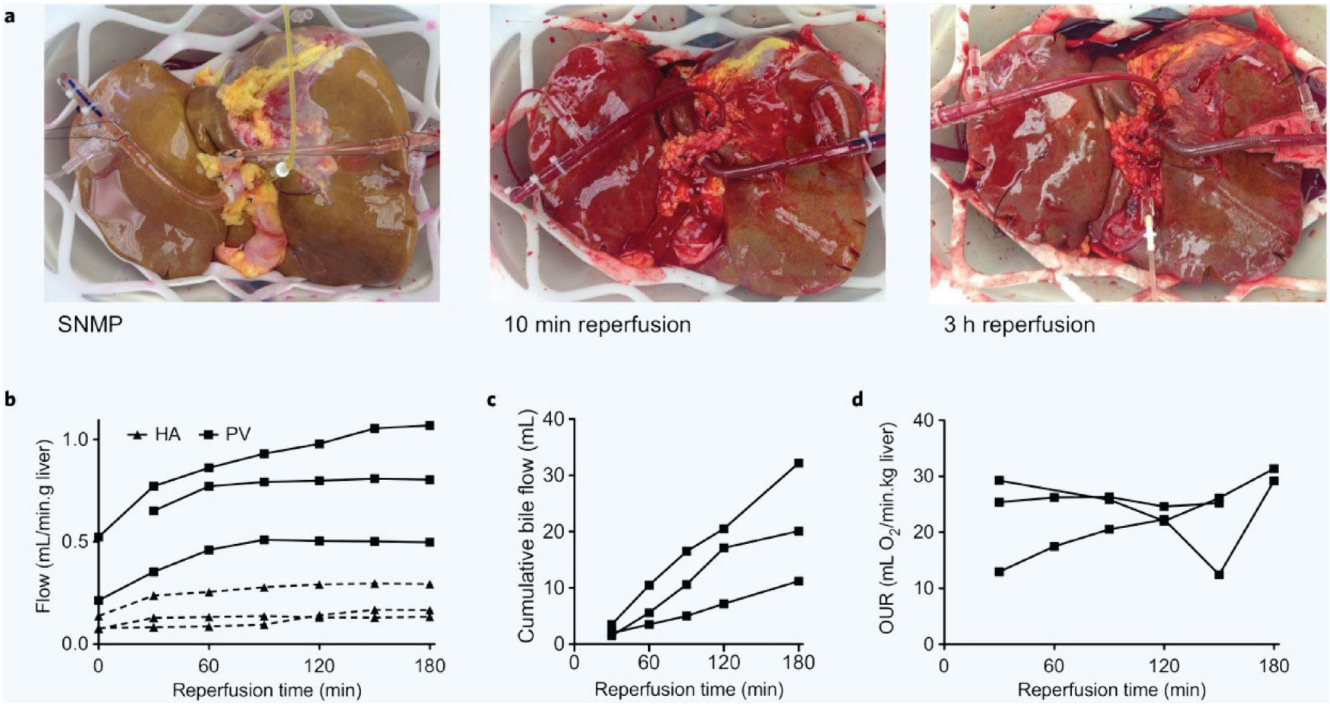

Figure 2. Reperfusion dynamics during simulated reperfusion.

Representative images of ex situ reperfusion of a human liver following preservation by subnormothermic machine perfusion (**a**), flow through the hepatic artery (HA) and portal vein (PV) during reperfusion (**b**), cumulative production of bile during reperfusion (**c**) and oxygen uptake from hemoglobin-bound and dissolved oxygen (**d**). Data is presented as an individual line per liver.

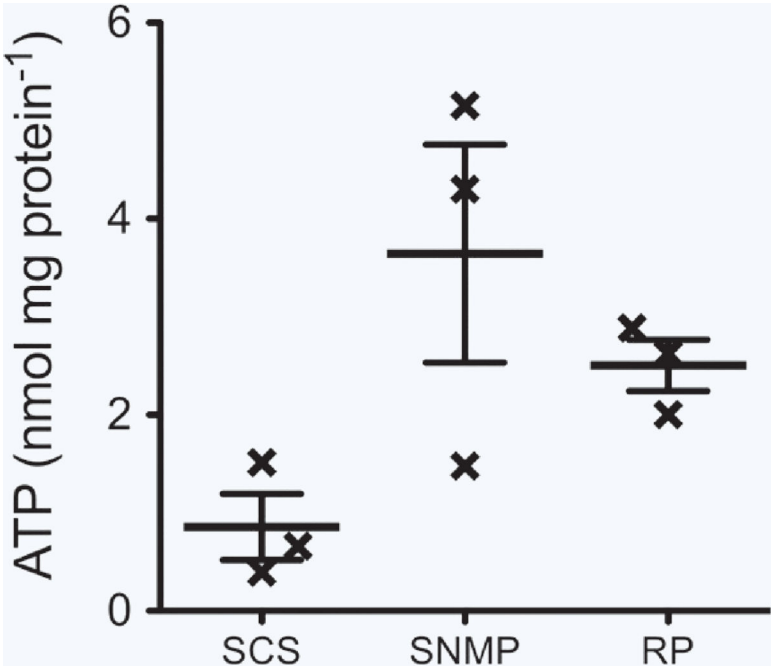

Figure 3. Liver tissue ATP content at various stages of preservation and reperfusion.

ATP content at the end of ~4 hours of static cold storage (SCS), at the end of subnormothermic machine perfusion (SNMP), and at the end of simulated reperfusion (RP). Bars represent mean + SE. Not significant; 2-way ANOVA.

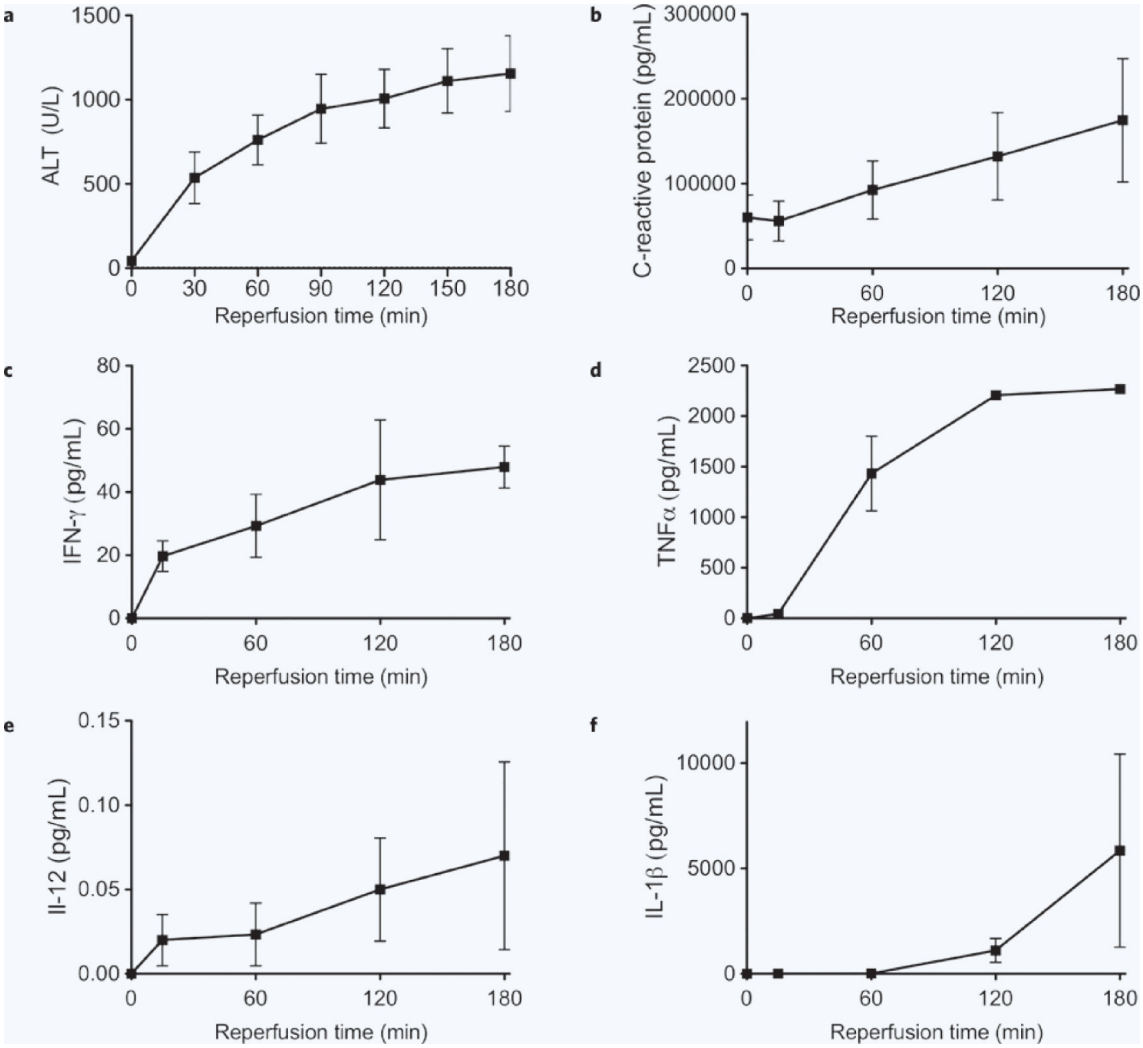

Figure 4. Markers of injury and inflammation during simulated reperfusion.

Levels of alanine transaminase (ALT) (**a**), C-reactive protein (CRP) (**b**), and inflammatory cytokines; IFN-γ (**c**), TNF-α (**d**), IL-12 (**e**) and IL-1β (**f**) measured in blood. Data presented as mean + SE.

Table 1

Discarded human liver donor characteristics.

	Age	Sex	Type	CIT	Donor WIT	HCV	Donor COD	Liver weight (g)
1	63	M	DCD	259	24	-	Head trauma	1840
2	33	M	DCD	254	13	-	Anoxia	2430
3	58	F	DCD	291	27	-	Head trauma	1620